sharply (Jackson, 1995). The clearance of the gut results in a characteristic amber colour of the infected hosts. The larvae may remain in this state for a prolonged period (1-3 months) before bacteria eventually invade the haemocoel, causing rapid death.

Please replace the paragraph on page 2, lines 9-14, with the following paragraph:

Another region involved in amber disease encoding was located by Nunez-Valdez and Mahanty (1996). They located a locus, *amb2*, by transposon mutagenesis and searching a cosmid genomic library. This region was chromosomally located and was involved in antifeeding in the larvae of *Costelytra zealandica*. However, the current applicant's research has demonstrated that the *amb2* region is located on pADAP remote from the virulence gene and is probably regulatory in function.

Please replace the paragraph on page 4, lines 1-8, with the following paragraph:

The invention further relates to an isolated nucleic acid molecule comprising a sequence of SEQ ID NO: 1, nucleotides 1955-18937 of SEQ ID NO: 1 or one or more of nucleotides 2411-9547, 9598-13884 or 14546-17467 of SEQ ID NO: 1, operably linked to at least one further nucleotide sequence which encodes an insecticidal protein. For example, the at least one further nucleotide sequence may be the nucleotide sequence which codes for the *Bacillus* delta toxins, vegatative insecticidal proteins (vips), cholesterol oxidases, *Clostridium bifermentens* mosquitocidal toxins and/or *Photorhabadus luminescens* toxins and so forth.

Please replace line 19 on page 5, with the following line:

The polypeptide may comprise amino acids 32-5112 of SEQ ID NO: 1.

Please replace the paragraph page 7, lines 6-8, with the following paragraph:

According to a further aspect the invention provides a method of inducing amber disease or like condition in insects comprising delivering to an insect an

effective amount of the polypeptide of the invention that has functional insecticidal activity against said insect.

Please replace the paragraphs on page 7, line 19, through page 8, line 3, with the following paragraphs:

The insecticidal polypeptide may be delivered to the insect orally either as a solid bait matrix, as a sprayable insecticide sprayed onto a substrate upon which the insect feeds, applied directly to the soil subsurface or as a drench or is expressed in a transgenic plant, bacterium, virus or fungus upon which the insect feeds, or by any other suitable method which would be obvious to a person skilled in the art.

According to further aspect, the invention provides a transgenic plant, bacterium, virus or fungus, incorporating in its genome, a nucleic acid molecule of the invention providing the plant, bacterium virus or fungus with an ability to express an effective amount of an insecticidal polypeptide.

Please replace the paragraph on page 9, lines 2-5, with the following paragraph:

In a further aspect, the present invention consists in replicable transfer vector suitable for use in preparing a polypeptide of the invention. These vectors may be constructed according to techniques well known in the art, or may be selected from cloning vectors available in the art.

Please replace the paragraph on page 9, lines 11-13, with the following paragraph:

Two major types of vectors possessing these characteristics are plasmids and bacterial viruses (bacteriophages or phages). Presently preferred vectors include pMOS-Blue, pGem-T and pUC8.

Please replace the paragraph on page 12, lines 6-9, with the following paragraph:

Preferably, such a nucleic acid construct is a vector comprising a replication system recognised by the host. For the practice of the present invention, well known compositions and techniques for preparing and using

vectors, host cells, introduction of vectors into host cells and so forth, are employed, as discussed, *inter alia*, in Sambrook et al (1989).

Please replace the paragraph on page 20, lines 3-7, with the following paragraph:

Table 1 lists bacterial isolates and plasmids used in the present invention. Bacteria were grown in LB broth or on LB agar (Sambrook et al. 1989), at 37° C for *Escherichia coli* and 30°C for *S. entomophila*. Antibiotic concentrations used (μg/ml) for *Serratia* were kanamycin 100, chloramphenicol 90, tetracycline 30 and for *e. coli* strains were kanamycin 50, chloramphenicol 30, tetracycline 15, and ampicillin 100.

Please replace the paragraph on page 23, lines 5-17, with the following paragraph:

Previously, Grkovic et al. (1995) have shown that the pADK-13 mutation can be complemented with the pADAP 11 kb *Hin*dIII fragment (gGLA-20). However, the pADK-10 mutation was unable to be complemented with pGLA-20. In an attempt to isolate the region that may complement the pADK-10 mutation the previously described pGLA-20 derived, pADK-35 null mutation (Grkovic et al. 1995) was used as a selective marker (Fig 1), to select the *Bgl*II fragment encompassing both the pADK-10 and pADK-35 mutations. pADK-35 DNA was isolated and digested with the restriction enzyme *Bgl*II. The resultant digest was ligated into the *Bam*HI site of bBR322 to form the construct pBG35 (containing 12.8kb *Bgl*II - mini-*Tn10* fragment). pBG35 was placed separately in *trans* with pADK-10 and pGLA-20, and the resultant strains bioassayed against grass grub larvae. Results showed that pBG35 was able to complement the pADK-10 mutant, but was unable to induce any symptoms of amber disease when placed in *trans* with pGLA-20, indicating that there must be another region on pADAP needed to induce amber disease.

Please replace the paragraph on page 26, lines 3-9, with the following paragraph:

The large *Bam*HI fragment (18937 bp) derived from the pBM32-8 was sequenced on both strands using a combination of constructed detections, plasmid subclones and custom made primers. A total continuous sequence of 18937 bp has been deposited in Gene Bank (Accession Number AF135182). Structural analysis of the DNA sequence using DNAMAN showed that there was a 12-bp sequence repeated five times between positions 683 and 743. The repeat is flanked by an upstream 13 base pair palindrome (669-682-bp), and a degenerate 34-bp downstream palindrome (765-799-bp)(Fig 2d,e).

Please replace the paragraph on page 31, line 18, through page 33, line 4, with the following paragraph:

The 23-kb region cloned into pBR322 to make pBM32 conferred pathogenicity in pADAP-cured *S. entomophilia* strains with all symptoms of amber disease being observed. Insertion mutants in pBM32 that abolished pathogenicity were transferred to pADAP. The resultant strains showed a partial disease phenotype, including anti-feeding but not gut clearance, suggesting that an additional anti-feeding gene may be present elsewhere on pADAP. The occurrence of two different anti-feeding genes on pADAP also supports data of Grkovic et al. (1995) who found that suppression of feeding was stronger in the wild-type pADK-6 strain, compared to the partial disease state (pADK-10, pADK-13) of inducing anti-feeding but no gut clearance. A putative anti-feeding gene, *amb2*, has already been isolated from the genomic DNA of *S. entomophilia* (Nunez-Valdez and Mahanty, 1996). Recent data indicates that the *amb2*, locus resides at an as yet to be identified location on pADAP that is remote from the region identified therein (Hurst, unpublished data).

Please replace the paragraph on page 36, lines 10-24, with the following paragraph:

Using the polymerase chain reaction (PCR) the initiation codons ATG of the three *sep* genes (*sepA, sepB* and *sepC*) were individually placed into the unique *Nde*I site (restriction enzyme site (CATGG) of the HIS-tag arabinose expression vector pAV2-10 (obtained from Chuck Shoemaker - AgResearch). Because large proteins i.e. greater than 50 kda are limited in their ability to bind to HIS tag affinity columns the carboxyl terminus of each of the Sep proteins did not need to be in frame with the HIS-tag site. Instead wild type DNA (non PCRd) containing a downstream chloramphenicol resistance gene was ligated into the appropriate restriction enzyme site (*sepA, Sun*I; *sepB Hin*dIII; *sepC Bst*XI) of the pAV2-10-*sep* derived vectors:-
-the use of the chloramphenicol resistant maker provided by the vector pACYC184 enhances the stability to each of the expression constructs i.e. -the antibiotic amphicilin to which the pAV2-10 is resistant too is cleaved in the media to an inactive form leading to possible plasmid free segregants arising. Conversely the antibiotic chloramphenicol is not cleaved heightening the level of plasmid stability under conditions of arabinose induction.

In the Claims:

Please replace claims 2-14, 16-33, 35-43 with the following claims (a marked-up copy of the amended claims is attached to this Amendment):

2. (Amended) A purified and isolated nucleic acid molecule as claimed in claim 1, comprising the nucleotide sequence 1995-18937 of SEQ ID NO: 1.

3. (Amended) A purfied and isolated nucleic acid molecule as claimed in claim 1, comprising one or more of the nucleotide sequences 2411-9547, 9589-13883 or 14546-17467 of SEQ ID NO: 1.

4. (Amended) A purified and isolated nucleic acid molecule as claimed in claim 3, comprising all of nucleotide sequences 2411-9547, 9598-13884 and 14546-17467 of SEQ ID NO: 1.

5. (Amended) A purified and isolated nucleic acid molecule as claimed in claim 1, comprising a sequence of SEQ ID NO: 1, operably linked to at least one further nucleotide sequence which encodes an insecticidal protein.

6. (Amended) A purified and isolated nucleic acid molecule as claimed in claim 2, comprising nucleotides 1955-18937 of SEQ ID NO: 1, operably linked to at least one further nucleotide sequence which encodes an insecticidal protein.

7. (Amended) A purified and isolated nucleic acid molecule as claimed in claim 3, comprising a sequence of SEQ ID NO: 1,or one or more of nucleotides 2411-9547, 9598-13884 or 14546-17467 of SEQ ID NO: 1, operably linked to at least one further nucleotide sequence which encodes an insecticidal protein.

8. (Amended Twice) A purified and isolated nucleic acid molecule of claim 4, wherein the sequence of nucleotides encodes at least one of the *Bacillus* delta endo toxins, vegatative insecticidal proteins (vips), cholesterol oxidases, *Clostridium bifementens* mosquitocidal toxins or *Photorhabadus luminescens* toxins.

9. (Amended) A purified and isolated nucleic acid molecule as claimed in claim 1, wherein nucleic acid molecule may comprise DNA, cDNA or RNA.

10. (Amended) A purified and isolated nucleic acid molecule as claimed in claim 1, wherein the nucleic acid molecules said fragment, neutral mutation or homolog thereof capable of hybridising to said nucleic acid molecule, hybridise to the nucleotide sequence of SEQ ID NO: 1, or nucleotides 1955-18937, 2411-9598-13884 or 14546-17467 of SEQ ID NO: 1 if there is at least 50%, preferably 60%, more preferably 70% and most preferably 90-95% or greater identity between the sequences.

11. (Amended) A purified and isolated nucleic acid molecule as claimed in claim 1, wherein the nucleic acid molecule may be isolated from *Serratia entomophilia* or *Serratia proteamaculans* strains of bacteria.

12. (Amended) A recombinant expression vector(s) containing the nucleic acid molecule as claimed in claim 1, and host transformed with the vector expressing a polypeptide.

13. (Amended) A recombinant expression vector(s) as claimed in claim 12, wherein the vector is selectable from any suitable natural or artificial plasmid/vector.

14. (Amended) A recombinant expression vector(s) as claimed in claim 13, wherein said suitable natural or artificial plasmid/vector, including pUC 19 (Yannish-Perron et al. 1995), pProEX HT (GibcoBRL, Gaithersburg, MD, USA), pBR322 (Bolivar et al 1977), pACYC184 (Chang et al. 1978), pLAFR3 (Staskowicz et al. 1987).

16. (Amended) A method of producing a polypeptide of claim 15, comprising the steps of:

(a) culturing a host cell which has been transformed or transfected with said vector as defined above to express the encoded polypeptide or peptide; and

(b) recovering the expressed polypeptide or peptide.

17. (Amended) A ligand that binds to a polypeptide of claim 15.

18. (Amended) A ligand as claimed in claim 17, wherein the ligand is an antibody or antibody binding fragment.

19. (Amended) Probes and primers comprising a fragment of the nucleic acid molecule as claimed in claim 1, wherein said fragment is hybridisable under stringent conditions to a native insecticidal gene sequence.

20. (Amended) Probes and primers comprising a fragment of the nucleic acid molecule as claimed in claim 19, wherein said probes and primers enable the structure and function of the gene to be determined and homologs of the gene to be obtained from bacteria other than *Serratia* sp.

21. (Amended) A polypeptide as claimed in claim 15, wherein the polypeptide has insecticidal activity encoded by the nucleic acid molecule of claim 1, or a functional fragment, neutral mutation or homolog thereof.

22. (Amended) A polypeptide having insecticidal activity as claimed in claim 21, wherein the polypeptide comprises the amino acid sequence of SEQ ID NO: 1 or a functional fragment, neutral mutation or homolog thereof.

23. (Amended) A polypeptide having insecticidal activity as claimed in claim 21, wherein the polypeptide comprises amino acids 32-5112 of SEQ ID NO: 1.

24. (Amended) A polypeptide having insecticidal activity as claimed in claim 21, wherein the polypeptide comprises at least one amino acid sequence of SEQ ID NO: 2; SEQ ID NO: 3; SEQ ID NO: 4; SEQ ID NO: 5 or SEQ ID NO: 6.

25. (Amended) A polypeptide having insecticidal activity as claimed in claim 24, wherein the polypeptide preferably comprises amino acid sequence SEQ ID NO: 4; SEQ ID NO: 5 and SEQ ID NO: 6.

26. (Amended) A polypeptide having insecticidal activity as claimed in claim 24, wherein the polypeptide preferably comprises all of SEQ NOs: 2-6.

27. (Amended) A polypeptide having insecticidal activity as claimed in claim 21, wherein the polypeptide is obtained by expression of a DNA sequence coding therefore in a host cell or organism.

28. (Amended) A polypeptide having insecticidal activity as claimed in claim 27, wherein the polypeptide comprises the amino acid sequence of SEQ ID NO: 1 linked to at least one further amino acid sequence encoding an insecticidal protein.

29. (Amended) A polypeptide having insecticidal activity as claimed in claim 28, wherein the at least one further amino acid sequence includes the amino acid sequence which codes for *Bacillus* delta endo toxins, vegatative insecticidal proteins (vips), cholesterol oxidases, *Clostridium bifermentens* mosquitocidal toxins and/or *Photorhabadus luminescens* toxins.

30. (Amended) A polypeptide having insecticidal activity as claimed in claim 28, wherein the polypeptides comprise at least 50%, preferably 60%, more preferably 70% and most preferably 90-95% or greater identity to SEQ ID NO: 1.

31. (Amended) A polypeptide having insecticidal activity as claimed in claim 21, wherein the polypeptide is produced by expression of a vector comprising the nucleic acid of SEQ ID No:1 or a functional fragment, neutral mutation or homolog thereof, in a suitable host cell.

32. (Amended) An insecticidal composition comprising at least the polypeptide as claimed in claim 21, and an agriculturally acceptable carrier.

33. (Amended) An insecticidal composition as claimed in claim 32, wherein more than one polypeptide is included in the composition.

35. (Amended) An insecticidal composition as claimed in claim 34, wherein the composition comprises other known insecticidally active agents, including *Bacillus* delta endo toxins, vegatative insecticidal proteins (vips), cholesterol oxidases, *Clostridium bifermentens* mosquitocidal toxins and/or *Photorhabadus luminescens* toxins.

36. (Amended) A method of combating pests, said method comprising applying to a locus, host and/or the pest, an effective amount of the polypeptide as claimed in claim 21, that has functional insecticidal activity against said pest.

37. (Amended) A method of inducing amber disease or like condition in insects comprising delivery to an insect an effective amount of the polypeptide as claimed in claim 21, that has functional insecticidal activity against said insect.

38. (Amended) A method of inducing amber disease or like condition in insects as claimed in claim 37, comprising delivery to an insect an effective amount of the polypeptide wherein the insect is selected from the order comprising Coleoptera.

39. (Amended) A method of inducing amber disease or like condition in insects as claimed in claim 38, comprising delivery to an insect an effective amount of the polypeptide wherein the insect includes *Costelytra zealandica* (Coleoptera: Sacarabaeidae).

40. (Amended) A method of delivering the insecticidal polypeptide to induce amber disease or like condition in insects including delivery of the insecticidal

polypeptide as claimed in claim 39, to the insect by a method selected from the group consisting of presenting the insecticidal polypeptide orally as a solid bait matrix, as a sprayable insecticide sprayed onto a substrate upon which the insect feeds, applied directly to the soil subsurface or as a drench or is expressed in an transgenic plant, bacterium, virus or fungus upon which the insect feeds.

41. (Amended) A transgenic plant, bacterium, virus or fungus, incorporating in its genome, a nucleic acid molecule as claimed in claim 1, for providing the plant, bacterium, virus or fungus with an ability to express an effective amount of an insecticidal polypeptide.

42. (Amended) A purified and isolated nucleic acid molecule of claim 5, wherein the sequence of nucleotides encodes at least one of the *Bacillus* delta endo toxins, vegatative insecticidal proteins (vips), cholesterol oxidases, *Clostridium bifementens* mosquitocidal toxins or *Photorhabadus luminescens* toxins.

43. (Amended) A purified and isolated nucleic acid molecule of claim 6, wherein the sequence of nucleotides encodes at least one of the *Bacillus* delta endo toxins, vegatative insecticidal proteins (vips), cholesterol oxidases, *Clostridium bifementens* mosquitocidal toxins or *Photorhabadus luminescens* toxins.